FOOD LEVEL IN RELATION TO RATE OF DEVELOPMENT AND EYE PIGMENTATION IN DROSOPHILA MELANOGASTER [1]

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Introduction

Beadle and Law (1938) have shown that the eye color hormones concerned in the differentiation of vermilion and cinnabar eye colors in *Drosophila melanogaster* are effective when administered with larval food. In attempting to develop a standardized method of feeding these hormones quantitatively, it became evident that information was needed on the relation between food-level and rate of development (see Northrop, 1917*a*, and Baumberger, 1919, for accounts of previous work bearing on this). During the course of experiments designed to give such information, Dr. Boris Ephrussi of the Institut de Biologie physicochimique, Paris, informed us (personal communications) of feeding experiments made in his laboratory, the results of which appeared to be understandable on the assumption that food-level had an effect on pigment production in genetically vermilion brown flies. After preliminary experiments had given results consistent with this assumption, systematic studies, approaching the general problem in somewhat different ways, were undertaken in the two laboratories. The paper preceding this (Khouvine, Ephrussi, and Chevais, 1938) and the present report summarize these experiments.

The Seventy-Hour Change

If larvae are removed from standard food (cornmeal-molasses-agar, seeded with an excess of fresh baker's yeast) at various stages of development, rinsed with Ringer's solution, and transferred to vials containing filter paper moistened with Ringer's solution, there is no further increase in size. Those removed at any time up to about 70 hours after egg-laying (at 25° C., the temperature at which all experiments were carried out) may continue to live for several days but eventually die unless given additional food. Larvae similarly removed from food shortly after 70 hours fail to increase in size but continue to differentiate

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and form puparia at approximately the same time as do normally fed controls (at from 100–120 hours after egg-laying, depending on the stock used). The results of a typical series of this kind in which lots of vermilion brown larvae were removed from full food to no food at successive 4-hour intervals are summarized in Fig. 1. In this series eggs were collected over a 2-hour period; the ages indicated are measured from the midpoint of the egg-collecting period. Results similar to these have also been obtained using larvae of the Oregon-r wild type stock.

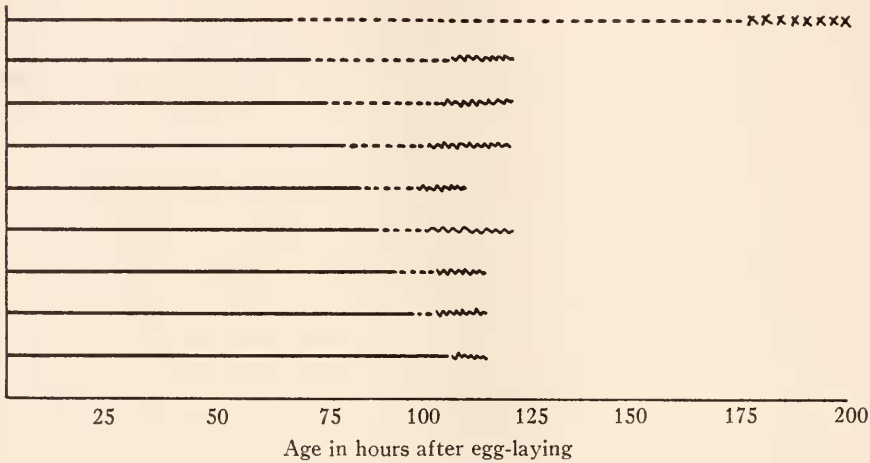

FIG. 1. The effect of removing vermilion brown larvae from food to moist filter paper at various times. Each horizontal line represents a sample of 20 individuals. Age in hours is represented on the horizontal axis. Unbroken lines represent time on full food, dotted lines time on filter paper, wavy lines puparium formation, and *x*'s represent death.

These results show clearly that some organizational change occurs in larvae at about 70 hours (near the time of the second moult). This will be referred to as the "70-hour change" and will be considered further in subsequent sections.

RESULTS OF COMPLETE STARVATION BEFORE THE SEVENTY-HOUR CHANGE

Several series of experiments have been made in which larvae at various ages were removed from food before the 70-hour change, kept on moist filter paper for varying intervals, and then returned to standard food medium with an excess of yeast. All series, some with the Oregon-r stock, others with a vermilion brown stock, were consistent in indicating that removal from food at from 24 hours after egg-laying

(some 7–8 hours after hatching from the egg) to just prior to the 70-hour change, resulted in cessation of those developmental processes concerned with differentiation. Larvae so removed from food were delayed in forming puparia, the delay being roughly proportional to the time of starvation. Since time of puparium formation was not recorded accurately (generally counts of puparia were made at 12-hour intervals), it is not considered worthwhile to record these data in complete form. The results of a typical series in which vermilion brown larvae were removed from food at 60 hours and returned in lots of 20 at 12-hour intervals are shown in Fig. 2. Other experiments included larvae

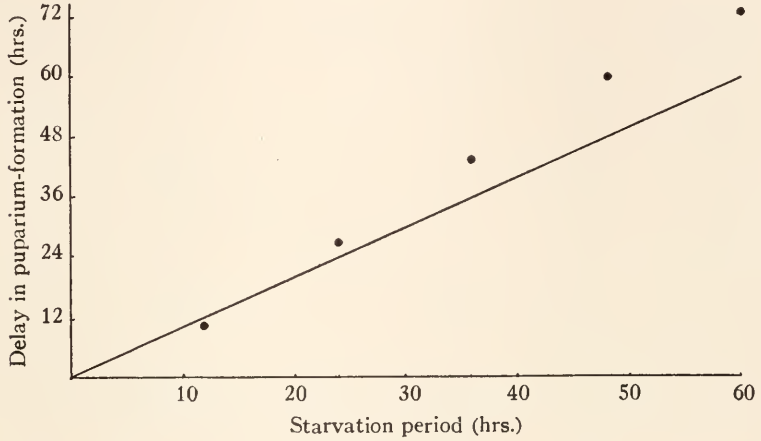

Fig. 2. Relation of delay in puparium formation to length of starvation period in vermilion brown larvae removed from food at 60 hours after egg-laying. The straight line, delay equals starvation period, is for reference.

initially removed at 24, 36, 48 and 60 hours, and returned in lots of 20 at 12-hour intervals up to the time of death. Under the conditions of the tests the maximum starvation periods were as follows:

Age at removal from food (*hrs.*)	Maximum period starved (*hrs.*)
24	48
36	36
48	60
60	60

All results were roughly similar to those shown in Fig. 2. The average delay in puparium formation in relation to time of starvation for four series of vermilion brown larvae, initially removed at 24, 36,

48, and 60 hours, is shown in Fig. 3. It is evident that the delay in puparium formation is generally greater than the time of starvation. These results suggest that under conditions of complete starvation the organizing processes which normally lead to metamorphosis are not only stopped but actually set back. Presumably materials already organized at the time of removal from food are broken down in the process of metabolism. After the restoration of an adequate food supply, these materials must be reorganized before the animal is back to the developmental stage attained at the onset of starvation. The data as obtained are not considered complete enough to justify an attempt to determine

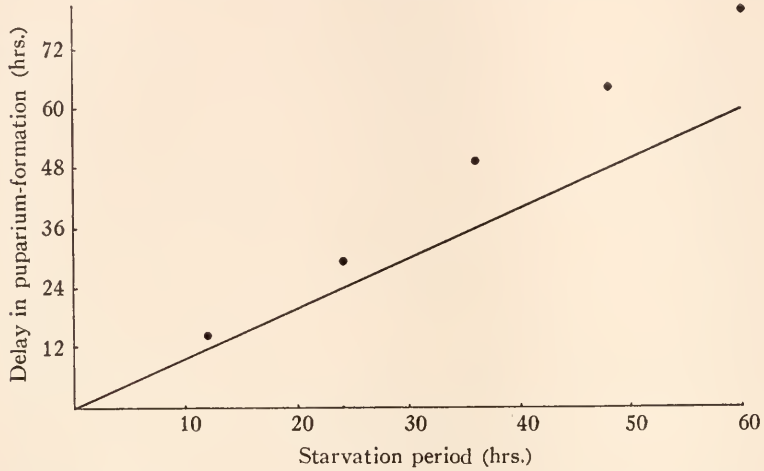

FIG. 3. Relation between average delay in puparium formation and length of starvation period, in four series of vermilion brown larvae, removed from food at 24, 36, 48 and 60 hours after egg-laying. The straight line, delay equals starvation period, is for reference.

the precise relation between delay in puparium formation and time of starvation.

Starvation prior to the 70-hour-change appears to be without effect on the duration of pupal life. The times from puparium formation to eclosion for four samples of vermilion brown flies subjected to periods of starvation were as follows:

Starvation period (*hrs.*)	Age at puparium formation (*hrs.*)	Time from puparium formation to eclosion (*hrs*)
None (control)	112	100
24 to 60	156	94
36 to 72	170	102
48 to 108	198	102
60 to 108	172	101

Considering the fact that both time of puparium formation and time of eclosion are approximations (observations made at 12-hour intervals), there is no evidence of any significant differences in length of pupal life. These observations agree with those reported by Northrop (1917*b*), Baumberger (1919) and by others.

Larvae subjected to complete starvation for various intervals of development prior to the 70-hour change and then returned to full food obtain full size by the time of puparium formation. Table I gives the oven-dry weights for adult flies from one series of vermilion brown

TABLE I

Weight of oven-dried adult flies from larvae subjected to starvation for various periods of development

Period starved (*hrs.*)	Total number of flies	Weight, av. ♀♀ and ♂♂ (*mg.*)
0	18	0.29
24–36	18	.26
24–48	18	.29
24–60	17	.31
24–72	7	.31
36–48	20	.27
36–60	19	.28
36–72	13	.25
48–60	19	.28
48–84	16	.34
48–96	8	.27
48–108	11	.28
60–72	19	.28
60–84	18	.26
60–96	18	.30
60–108	9	.29
60–120	3	.30

larvae. It is evident that there is no consistent effect of the treatment on the final weights.

The Effects of Periods of Complete Starvation of Larvae After the Seventy-Hour Change

As indicated above, removal of larvae from food to moist filter paper after the 70-hour change does not result in a delay in puparium formation. In some series an actual acceleration of puparium formation, amounting to several hours, appeared to result from removal from food. The data obtained, considered above, are probably not sufficient to establish definitely such acceleration as a real effect of the starvation. These

observations are, however, in agreement with the results of intermittent feeding on the development of the moth *Lymantria* (Kopeć, 1924). In this insect, more suitable for such studies because of the longer life cycle, starvation in late larval stages clearly brought about a hastening of pupation.

Adults from larvae removed from food shortly after the 70-hour-change are much smaller than fully-fed flies otherwise comparable. From a collection of vermilion brown larvae from eggs laid during a two-hour period, lots of 20 larvae were removed to moist filter paper

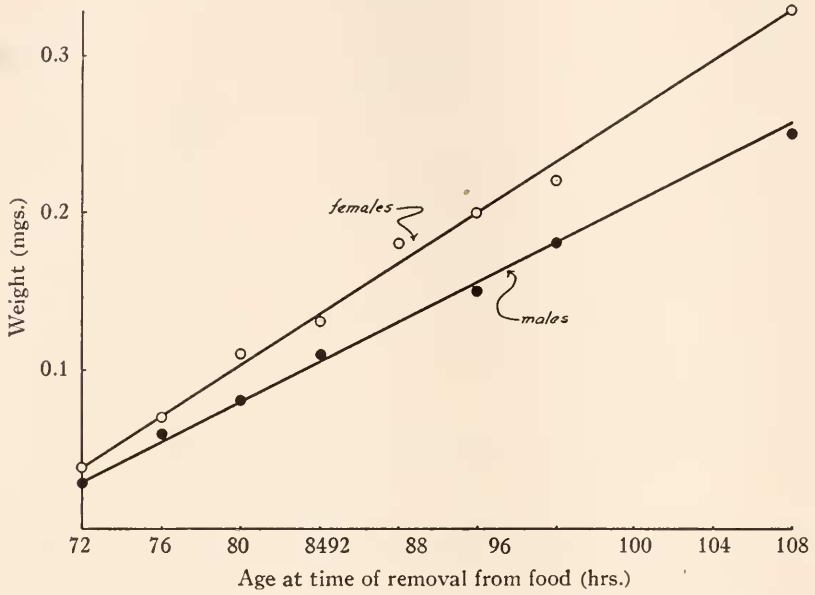

FIG. 4. Relation between oven-dry weight of adult flies from vermilion brown larvae removed from full food at successive 4-hour intervals and allowed to complete development on moist filter paper.

at the 4-hour intervals (represented in Fig. 1). The adult flies from these larvae were oven-dried at about 110° C. until they no longer showed a decrease in weight, and their weights recorded. Males and females were weighed separately. The weight of flies (average males and females) from larvae removed from food at 72 hours was only 0.035 mg. each or only about one-eighth as much as fully fed controls which weighed 0.290 mg. each. The relation of final weight to time of removal from food is shown in Fig. 4. It is clear that size as determined by weighing is roughly proportional to the time after the 70-hour-change during which the larvae are allowed to feed. Whether or not

the relation is linear, as Fig. 4 might suggest, the data are not sufficient to determine.

Aside from their size, these flies, starved from 72 hours, appeared to be normal. Tests made by intercrossing the small flies resulting from complete starvation of larvae after 72 hours showed that both sexes were fertile; no attempt was made to mate such small flies to flies of normal size.

In general, the same effects of starvation as are considered above in *Drosophila* were observed in *Lymantria dispar* by Kopeć (1924). Kopeć discusses in some detail the significance of such results as these to the more general problems of development and differentiation.

Attempts to Induce the Seventy-Hour-Change

On the assumption that specific organizing substances might be concerned in the 70-hour change, extracts of animals that had undergone this change were injected into larvae removed from food before 70 hours. Wild type larvae, taken shortly before puparium formation, were ground with powdered silica in an ether-alcohol mixture. The liquid was decanted, evaporated by gentle warming, the residue taken up in water, and centrifuged. The oily extract obtained gave negative results when injected into vermilion brown larvae removed from food at 68 hours (15 test larvae died without forming puparia). The water extract obtained by the above procedure was similarly tested, and of 15 test animals, one pupated. In the uninjected control animals, however, one in 45 also pupated, so that there was no definite indication of a positive effect.

Tests were made of fluid obtained by crushing and centrifuging wild type pre-pupae with white puparia, i.e., larvae that have formed puparia within an hour. The water layer was negative when tested in vermilion brown larvae taken from food at 68 hours. The results were the same with fluid from unheated and heated (100° C. for about 1 minute) pre-pupae (12 tests with heated and 14 with unheated material).

Preliminary experiments indicated that a ring gland (Hadorn, 1937) from a 100 ± hour larvae transplanted to larvae removed from food at 68 hours would induce puparium formation and pupation at the time that fully fed controls underwent these changes. Additional tests of the same type gave results that were not convincing. Of sixteen 68-hour test animals injected with ring-glands from 100 ± hour vermilion brown larvae, 15 lived and of these 6 formed puparia but did not continue to develop. Of a similar number of surviving controls injected with Ringer's solution, 2 formed puparia and likewise failed to continue development. In another test, 3 of 10 animals to which ring glands had

been transplanted formed puparia and failed to continue development. In this test all 17 controls injected with Ringer's solution failed to form puparia. It appears from these results that the ring gland may exert some influence on organization processes, but this is by no means proved. Furthermore, assuming such an effect, the possibility has not been excluded that it is simply a question of adding more potential food material; that is, the addition of a ring gland might be roughly equivalent to feeding the larvae for a few hours.

Clearly more information is needed before an attempt to interpret the nature of the 70-hour change in specific terms is likely to be profitable.

Relation of Food Level to Eye Pigment Development in Vermilion Brown Flies

Preliminary trials showed that by taking larvae of apricot vermilion or vermilion brown flies and placing them under conditions of reduced food, a darkening of the eyes could be induced. The experiments reported below, all made with vermilion brown flies, were designed to find out more about this starvation effect.

Conditions appropriate for producing a modification in the eye color of vermilion brown flies have no detectable effect on the eye color of cinnabar brown flies.

Effects of Complete Removal of Food

In the series of experiments reported in previous sections of this paper, in which vermilion brown larvae were removed from food to filter paper at different ages and completely starved for varying lengths of time, careful observations were made of the eye colors of the adult flies obtained. The results were consistently negative. Complete removal from food was without apparent effect on the eye color regardless of the age of the larvae at the beginning of the starvation period and of the duration of the starvation period. Intermittent feeding gave similar negative results. In one such series 44 larvae, initially removed from food at 36 hours after egg-laying, were treated as follows:

Time on food (*hrs.*)	Time off food (*hrs.*)
Hatching to 36	36 to 72
72 to 88	88 to 109
109 to 121	121 to 135
135 to 216 (puparium formation)	

With this treatment, purposely made drastic, the mortality was high; only two flies survived. These showed no modification of the eye color.

Other experiments of essentially the same nature likewise gave negative results.

Effects of Intermediate Food Level

It was found in one experiment that vermilion brown larvae completely starved from 48–96 hours, then fed a few drops of yeast suspension on filter paper, as the yeast became exhausted gave rise to adults with eyes definitely darker than the controls. Four flies eclosed on the twelfth day after egg-laying were only slightly modified, 13 that emerged on the thirteenth day gave a maximum color value of 1.0 (0.0 represents no modification, 5.0 a complete change to brown; see Tatum and Beadle, in press, for details concerning eye color standards). This and similar experiments suggested, then, that an intermediate food-level was effective in inducing a modification of the processes concerned with eye pigment formation.

To determine the possible influence of varying food levels on the eye color of vermilion brown flies, vials were made up containing 10 cc. of 1 per cent brewer's yeast in 2 per cent agar. Varying numbers of larvae were removed from full food at 25 hours after egg-laying and placed in such vials. It was found that mortality increased as food level decreased, from 0 per cent with 10 larvae per vial to 50 per cent with 100 larvae per vial. Delay in puparium formation increased as the amount of food decreased, and, in general, size of adult flies decreased with decrease in food available per larva. The effect on eye color was marked, as indicated in Table II. A general relation was observed between size of adults and strength of modification; i.e., smaller flies were darker.

The data in Table II suggest that there is a food level between the two extremes represented in the experiment at which the modification in eye color is at a maximum. That there should be such an optimum food level for inducing this response is, of course, obvious from the fact that the response can be induced and that the two extremes, full food and no food, are ineffective.

A second experiment of the same nature but using 10 cc. of 1 per cent agar containing 0.5 per cent brewer's yeast, gave essentially similar results. As expected, the larval mortality was higher, dropping from 20 per cent with 10 larvae per vial to 80 per cent with 100 larvae.

Period of Development Sensitive to Low Food Levels

In order to bring about a modification of the eye color of vermilion brown flies by means of reduced food, the larvae must be put at a low food level before a certain period of larval development. Table III

Table II

Results of different food levels on the eye color of vermilion brown. Varying numbers of larvae were transferred at 25 hours after egg-laying (7–8 hours after hatching) to vials containing 10 cc. of 2 per cent agar agar with 1 per cent of dry brewer's yeast. Results are recorded in arbitrary color values followed in parenthesis by the number of individuals on which the color value is based. Fully fed controls showed no modification of eye color.

Number of larvae per vial	Delay in eclosion as compared with fully fed controls—days				
	1	2	3	4	5
10 20 30*	1.6 (42)	2.8 (7)			
40*	1.9 (19)	3.2 (4)			5.0 (1)
50*	1.9 (17)	2.1 (7)	4.3 (3)	4.2 (2)	
100		2.0 (18)			

* Thirty flies from the 30-, 40-, and 50-larva vials were combined. These eclosed 2 days later than controls and gave a color value of 2.8 (2.0–4.0).

gives the results of an experiment in which vermilion brown larvae of various ages were removed from full food to a low food level. Larvae transferred at any time up to and including 63 hours after egg-laying showed a relatively strong modification of eye color. Transferred at 75 hours or later, the result is quite negative. Within the limits of the experiment, this period, after which the eye color change no longer results, coincides with the 70-hour change. This result was confirmed in a second series not reported here in detail.

Table III

Effect on eye color of removing vermilion brown larvae from full food at different ages and placing them on a low-food level. The low-food medium consisted of 10 cc. of 2 per cent agar containing ¼ per cent of air-dried brewer's yeast per vial. Twenty larvae were placed in each vial. Ages are measured from egg-laying.

Age at transfer to low food	Age at return to full food	Number of adult flies	Color value, mean and range
(*hrs.*)	(*hrs.*)		
28	175	11	3.7 (2.8–4.5)
39	175	19	3.4 (2.0–4.0)
51	175	10	2.7 (1.0–3.5)
63	175	14	3.3 (2.0–4.5)
75	Not returned	20	0.0
87	Not returned	18	0.0
99	Not returned	19	0.0
Control		17	0.0

The transfer to low food appears to be no more effective if made early in development than if made at 63 hours (Table III). In other words, it appears that the period primarily sensitive to the treatment lies between the ages of 63 and 75 hours. In order to test this further, experiments were made in which vermilion brown larvae transferred to low food about 10 hours after hatching from the eggs were returned to full food at intervals. The results of such an experiment are presented in Table IV. Subjection to a low food level early in development has very little effect. If the larvae are left on the low food medium until 87 hours after egg-laying the modification is very much stronger. Of course this chronological age of 87 hours does not correspond to a similar developmental age. By subtracting from the chro-

TABLE IV

Effect on eye color of returning vermilion brown larvae grown at a low food level (as described in heading to Table III) from 28 hours after egg-laying to full food.

Age at return to full food	Approximate delay in pupation	Number of adult flies	Color value, mean and range
(*hrs.*)			
39	8	10	0.0
51	15	17	0.2 (0.0–0.5)
63	18	12	0.2 (0.1–0.3)
75	18	17	0.3 (0.2–0.5)
87	24	14	1.8 (0.2–2.5)
99	36	14	0.7 (0.1–2.0)
Control	Full food throughout	17	0.0

nological age the delay in puparium formation we get a rough approximation of the developmental age. It is found by doing this that, within the limits of accuracy of the experiment as carried out, this type of experiment gives results consistent with the reciprocal one just described; that is, to obtain marked modifications of eye color, larvae must be subjected to a low food level during a period corresponding to that in the region 60 to 70 hours of normal development.

If larvae are kept on full food up to 60 hours after egg-laying, transferred to a low food medium and returned at intervals to full food, it is found that the modification in eye color increases with increasing time on low food. The results given in Table V show this. The maximum effect is produced in the lot of larvae kept on low food for 66 hours. The delay in puparium formation and eclosion (only roughly measured)

indicates that during this time they had advanced developmentally some 16 hours or less.

The above results are in general agreement in showing that the period sensitive to low food level as measured by the production of eye pigment lies between the stage reached at about 60 hours at full food and the 70-hour change, and that the maximum response is obtained when all of this developmental period is gone through on a low food level.

Relation of Food Level to the Eye Color Hormone System

Since it is known that the addition of a hormone-like substance to a vermilion fly will result in the production of an eye color like that of wild type (see Ephrussi, 1938, for review and references to literature), an

TABLE V

Influence of length of time on low food level on the eye color of vermilion brown flies the larvae of which were transferred from full food to low food at 60 hours after egg-laying. Low food has the same significance here as in Table III.

Age at return to full food	Time at low food level	Number of adult flies	Color value, mean and range
(*hrs.*)			
69	9	19	0.0 (0.0–0.5)
77	17	17	0.1 (0.0–0.4)
84	24	18	0.7 (0.0–3.3)
94	34	15	1.3 (0.4–1.7)
101	41	13	0.8 (0.4–2.0)
126	66	10*	3.1 (1.0–4.3)

* Approximate delay in eclosion as compared with controls, 50 + hours.

attempt was made to determine whether or not the increase in eye color intensity caused by partial starvation resulted from the production of increased amounts of v^+ hormone by such flies. Approximately 1700 vermilion brown larvae were grown under conditions calculated to give a reasonably strong modification in eye color. These were allowed to pupate and some 20–60 hours after puparium formation they were collected, cleaned and oven-dried at 100° C. In this way 0.69 gram of dry material was collected. A sample of 9 such flies allowed to develop gave a mean color value of 2.3 (1.5–3.0). As a control 4.5 grams of oven-dried vermilion brown pupae, fully fed as larvae, were collected. A sample allowed to develop gave a color value of 0.0 as expected. Crude extracts of these two lots of material were prepared according to the method described by Tatum and Beadle (in press). This extract in

a rather concentrated form was tested in the usual way by injection into vermilion brown test animals. From the tests of the extract of the starved vermilion brown pupae, it can be calculated that at the same concentration as the non-starved control this extract would have given a maximum color value of 1.6 (range 0.8–1.6). The reduced food level therefore resulted in an increased production of v^+ hormone by vermilion brown flies. From the results reported by Tatum and Beadle, we can calculate that an extract of wild type pupae of a concentration equal to that of the non-starved vermilion brown pupae would contain about 112 units of hormone per injection. Since one-third of a unit per injection can be detected without difficulty, the hormone content of the non-starved vermilion brown pupae can be said to be less than one three hundred and thirty-sixth that of wild type. The amount of hormone extracted from the partially starved vermilion brown pupae amounts to approximately 14 per cent of that extractable from wild type pupae. The relation between eye color and amount of hormone available per fly, given by Tatum and Beadle, indicates that at least 30 units are normally produced by a wild type fly. The extraction method used yields about 10 units per wild type fly. Assuming this same ratio of amount extracted to the amount produced to hold for the vermilion brown flies grown on reduced food, we can calculate that these flies would have produced an average of about 4.2 units per fly. This corresponds to an eye color value of 2.7 which is as close as could reasonably be expected to the observed color value of 2.3.

The magnitude of the increase in v^+ hormone resulting from reduced food can be approximated in the following manner: assuming that a vermilion brown fly does produce some v^+ hormone, we know that this amount must be less than one-third of a unit as defined by Tatum and Beadle. The maximum effect produced by growing vermilion brown larvae on a low food level is a color value of 5 which corresponds to an injection of at least 30 units of hormone. It follows, therefore, that the hormone is increased at least a hundredfold by subjecting vermilion brown larvae to the appropriate food level.

Discussion

From the results presented above it is seen that the conditions resulting in what might be called the "starvation effect" on the eye color of vermilion brown flies are accompanied by a retardation in development. That this retardation is, in itself, not responsible for the effect is shown by the fact that larvae retarded by complete removal from food do not show it. Furthermore, experiments not reported above have shown that retardation during the sensitive period brought about

by subjecting larvae to a temperature of 15° C. is quite without effect. The final size attained by the fly has nothing essential to do with the effect since larvae returned to full food after the effective period of the treatment are normal in size and may show strong modification of eye color.

A possible explanation of the effect of food level on the eye color change that we are concerned with involves the assumption that the effective low food levels may affect developmental reactions differentially. It might be supposed, for example, that the reactions leading to the formation of v^+ hormone go on slowly in a vermilion fly and that by prolonging development other reactions may be retarded and at the same time the conditions remain favorable for the formation of v^+ hormone. The magnitude of the effect, however, is such that this interpretation seems improbable. As pointed out above, the increase in hormone is at least one hundred times that in fully fed vermilion brown flies. With no change in rate, we would then have to assume an increase in time during which a reaction goes on of at least this magnitude. Since the delay accompanying the maximum response is less than 100 hours, the normal time required for the reaction assumed to be prolonged by the treatment would be less than one hour. While this simple interpretation, of course, is conceivable, it does not seem probable, especially in view of the fact that the sensitive period appears to extend over a period corresponding to about 10 hours of normal development.

Although the mechanism by which the starvation effect is brought about is unknown, it is suggested, as an hypothesis, that the food level effective in bringing about the eye color change modifies metabolism in such a way that the products normally used in one manner in a vermilion fly are utilized in the formation of v^+ hormone. The hormone involved is known from the work of Khouvine and Ephrussi (1937) and that of Tatum and Beadle (in press) to be amino acid-like in nature. It seems reasonable, therefore, to suppose that amino acids of one kind or another are the precursors of this hormone and that conditions are such in a vermilion fly that these precursors are utilized largely in protein synthesis rather than in the production of hormone. The conditions of low food that result in the formation of an appreciable amount of hormone are such that protein breakdown, localized or general, might be expected to result, and it seems possible that intermediate products of this breakdown, possibly amino acids, could be diverted to reactions leading to hormone synthesis. For further discussion of this question see the accompanying paper by Khouvine, Ephrussi and Chevais.

On first thought it seems strange that the starvation effect should not have been noted previously in stock bottles. It is, of course, a well-

known fact that under the normal conditions of *Drosophila* culture, the larvae are not optimally fed. Observations of crowded stock bottles made for the express purpose of determining whether or not vermilion brown flies showed the modification under these conditions, were negative. Presumably under standard culture conditions the drop in the amount of food available is rapid with the growth of larvae and the intermediate level at which the effect becomes strong is maintained for a relatively short time. The experiments reported above show that the appropriate food level must be maintained for some 36 hours and that this level must coincide with the sensitive period of development. Thus it appears that the conditions that result in the starvation effect are not likely to be met in standard culture techniques.

It is evident from the results reported here and in the preceding paper by Khouvine, Ephrussi and Chevais that the detection or measurement of v^+ hormone by the feeding technique may be complicated by the so-called starvation effect. From the fact that larvae older than 70 hours (full food at 25° C.) no longer show the eye color change associated with reduced food, but still react to v^+ hormone in the food medium, it is clear that feeding tests made with larvae transferred from full food after 70 hours are not subject to this complication.

Summary

In the development of larvae of *Drosophila melanogaster* an organizational change takes place at about 70 hours after egg-laying (25° C.). This change is referred to as the "70-hour change."

Larvae deprived of all food for periods at any time up to the 70-hour change are retarded in development, the retardation being a function of the length of the starvation period.

Larvae deprived of food after the 70-hour change are not retarded, but are probably somewhat accelerated in development.

If deprived of food during a portion of the larval developmental period after the 70-hour change, the final size attained by the fly appears to depend largely on the proportion of this period spent on full food.

Periods of complete removal of food from vermilion brown larvae are without apparent effect on eye pigment production. This is true whether the starvation period be short or long and whether it comes before or after the 70-hour change.

Partial starvation (at intermediate food levels) of larvae, under certain conditions, greatly increases the amount of eye pigment formed by vermilion brown flies.

The period of development most sensitive to this "starvation effect" on pigment production by the eye, lies shortly before the 70-hour change.

The production of a supposedly specific eye color hormone, v^+ hormone, can be influenced greatly by the amount of food given to vermilion brown larvae during a limited period of development, the period of maximum sensitivity referred to above. This increase is not less than a hundredfold.

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